Host Range of Tomato Mottle Virus, a New Geminivirus Infecting Tomato in Florida

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ABSTRACT

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A geminivirus causing mottling, upward leaf curling, and stunting was observed infecting tomatoes (*Lycopersicon esculentum* Mill. var. *esculentum*) throughout production areas of Florida since 1989; and it has been named the tomato mottle virus (TMoV). The virus was inoculated by whiteflies (*Bemisia tabaci* (Gennadius)) to 41 plant species representing eight families. Species of four genera became infected, three in the Solanaceae (*Lycopersicon, Nicotiana,* and *Physalis*) and one in the Fabaceae (*Phaseolus*). The infection in *Phaseolus vulgaris* L. was symptomless and was identified by nucleic acid spot hybridization with a full-length B component probe and by back inoculation to tomato by whiteflies. TMoV resembled other tomato-infecting geminiviruses from the Western Hemisphere in its narrow host range, in which species of the Solanaceae were predominate, but differed in the type of symptoms produced in tomato and in the species of hosts which were infected. Transmission via tomato seed was not found in 3,000 seedlings examined.

An epidemic of a virus, later named tomato mottle virus (TMoV) (Geminiviridae), was first observed in Naples, Florida, in the spring tomato crop of 1989 (J. K. Brown, *personal communication*) and was later found in all tomato production areas of Florida. The virus can occur whenever tomato plants are present, with incidences reported as high as 95% (1,8,9,17). TMoV was conservatively estimated to have reduced the value of the 1990–91 southwestern Florida tomato crop by $125 million (D. J. Schuster, *unpublished*). TMoV epidemics began after large populations of the whitefly (*Bemisia tabaci* (Gennadius)) biotype B began appearing in tomatoes (16). This new whitefly biotype was first observed in the fall of 1988. Before the introduction of the new biotype, *B. tabaci* occurred throughout much of Florida but was infrequently seen in tomato. TMoV was determined to be transmitted by *B. tabaci,* and inclusion bodies associated with whitefly-transmitted geminiviruses were observed in virus-infected tomatoes (8,9,17). This study was undertaken to determine a host range of TMoV as part of the characterization of the virus, to compare this host range with those of other tomato-infecting geminiviruses, and to identify other crop plant hosts which could influence the epidemiology of TMoV.

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MATERIALS AND METHODS

Sources of virus, whiteflies, and plants. The TMoV culture used in this study was obtained from a sample of infected tomato plants from the southwest area of Florida in 1989. This culture was the same as that used to determine the sequence of TMoV (1). The TMoV DNA clones produced from this source did not suggest a mixed infection was present (A. Abouzid, *personal communication*). The TMoV culture was maintained in tomato, *Lycopersicon esculentum* Mill. var. *esculentum* 'Florida Lanai', by whitefly transmission. Virus-infected tomato plants were maintained in aluminum- and organdy-screened cages (61 × 61 × 61 cm) in a 3 × 5 m fiberglass greenhouse.

The whiteflies used in this study came from a colony started in 1990 from greenhouse poinsettia (*Euphorbia pulcherrima* Willd. ex Klotzsch) and subsequently maintained on poinsettia in an indoor insect rearing room. A sample of 20 whitefly adults from this colony was collected, and all individuals were identified as *B. tabaci* biotype B using isoenzyme analysis of phosphoglucomutase (courtesy of T. M. Perring, Riverside, CA).

The plants used in this study were grown from seed, except for *E. pulcherrima,* crown-of-thorns (*Euphorbia milii* Des Moul.), and honeysuckle (*Lonicera japonica* Thunb.), which were propagated from cuttings, and potato (*Solanum tuberosum* L.), propagated from seed pieces. Plants generated from cuttings were screened for geminivirus using a nucleic acid spot hybridization assay (NASHA, described below) before being inoculated with TMoV.

Seed transmission study. Seeds were collected from the fruit of six cv. Sunny tomato plants which had been inoculated with TMoV by whiteflies. Seeds were not treated with trisodium orthophosphate (TSP), which would have destroyed any virus on the surface of the seed. Tomato seeds were planted in containerized planter flats, two to three seeds per tray cell, in two trials. In the first trial, 2,000 seedlings in the planter flats were covered by organdy cloth to prevent inoculation by viruliferous whiteflies. In the second trial, 1,000 seedlings were maintained in screened cages. The germination rate was greater than 90% for both trials. Seedlings were assayed at the three to four true leaf stage. Two leaves were collected from each seedling and assayed for virus in groups of six to eight leaves by NASHA. In the case of any positive reactions, seedlings contributing tissue to the spot were assayed again separately.

Inoculation of test plants with TMoV. Pots containing test plants were covered with cylindrical whole plant cages made of 0.01-mm cellulose acetate with organdy-screened side openings and tops. Each cage was 10 cm in diameter and 30 cm in height. Usually each pot contained one test plant, except for plants producing very small seedlings, such as alfalfa (*Medicago sativa* L.), *Rhynchosia minima* (L.) DC., *Macroptilium lathyroides* (L.) Urb., and *Euphorbia heterophylla* L., where three to five plants were present in each pot. Test plants were inoculated with 20 whiteflies per cage. Two sets of controls were used, one consisting of test plants with nonviruliferous whiteflies and the other of plants with no whiteflies. Whiteflies acquired virus from TMoV-

Table 1. Experimental host range of tomato mottle virus (TMoV) as determined by whitefly (*Bemisia tabaci*) transmission

Test plant[a]	Common name	Symptoms[b]	NASHA[c]
Brassicaceae			
Brassica oleracea L. var. *capitata* L.	Cabbage	NS	−
Caprifoliaceae			
Lonicera japonica Thunb.	Honeysuckle	NS	−
Compositae			
Carthamus tinctorius L.	Safflower	NS	−
Helianthus annuus L. 'Florida Teddy Bear'	Sunflower	NS	−
Cucurbitaceae			
Cucurbita pepo L. 'Table Ace'	Squash	NS	−
Euphorbiaceae			
Euphorbia heterophylla L.	Painted leaf	NS	−
Euphorbia milii Des Moul.	Crown-of-thorns	NS	−
Euphorbia pulcherrima Willd. ex Klotzsch	Poinsettia	NS	−
Fabaceae			
Arachis hypogaea L.	Peanut	NS	−
Glycine max (L.) Merr.	Soybean	NS	−
Macroptilium lathyroides (L.) Urb.	Phasibean	NS	−
Medicago sativa L.	Alfalfa	NS	−
Phaseolus acutifolius A. Gray	Tepary bean	NS	−
Phaseolus coccineus L.	Scarlet runner bean	NS	−
Phaseolus limensis Macfady	Lima bean	NS	−
Phaseolus vulgaris L. 'Topcrop'	Common bean	NS	+
Rhynchosia minima (L.) DC.	Rhynchosia	NS	−
Vigna radiata (L.) R. Wilcz.	Mungbean	NS	−
Malvaceae			
Althaea rosea Cav.	Hollyhock	NS	−
Abelmoschus esculentus (L.) Moench 'Annie Oakley'	Okra	NS	−
Gossypium hirsutum L. 'Delta Pine 70'	Cotton	NS	−
Sida acuta J. Burm.	Teaweed	NS	−
Solanaceae			
Capsicum annuum L. 'Yolo Wonder'	Bell pepper	NS	−
Capsicum chinense Jacq.	Scotch bonnet	NS	−
Datura stramonium L.	Jimson weed	NS	−
Lycopersicon cheesmanii Riley f. *minor* (Hook. f.) Mull. PI 365896		LC,YM	+
Lycopersicon chilense Dun. LA 2930		YM	+
LA 2749		YM	+
Lycopersicon esculentum Mill. var. *esculentum* 'Sunny'	Tomato	MO,LC,S	+
'Florida Lanai'	Tomato	mMO,LC,S	+
Lycopersicon hirsutum Humb. & Bonpl. f. *glabratum* Mull. PI 126449		YM	+
Lycopersicon pennellii (Corr.) D'Arcy var. *pennellii* LA 716		YM	+
Lycopersicon peruvianum (L.) Mill. var. *peruvianum* PI 129152		YM	+
var. *glandulosum* PI 126443		YM	+
var. *dentatum* PI 127830		YM	+
Lycopersicon pimpinellifolium (L.) Mill. PI 379059		LC,MO,YM	+
Nicotiana benthamiana Domin.		MO,LC,S	+
Nicotiana edwardsonii Christie & Hall		MO,mLC	+
Nicotiana tabacum L.	Tobacco		
'Burley'		MO,LC	+
'V20'		MO,LC	+
'Xanthi'		MO,LC	+
Petunia × *hybrida* Hort. Vilm.-Andr.	Petunia	NS	−
Physalis wrightii A. Gray	Wright groundcherry	IC,S	+
Physalis alkekengi L.	Chinese lantern	LC,S	+
Physalis ixocarpa Brot. ex DC.	Tomatillo	YM,LC,S	+
Solanum melongena L. var. *esculentum* Nees	Eggplant	NS	−
Solanum tuberosum L.	Potato	NS	−

[a] Minimum of four test plants per species or cultivar inoculated with TMoV by groups of 20 to 40 *B. tabaci* given a 48-hr acquisition access period on source plants and a 48-hr inoculation access period on test plants.

[b] IC = interveinal chlorosis, LC = leaf curl, m = mild, M = mosaic, Mo = mottle, NS = no symptoms, NT = not tested, S = stunting, and YM = yellow mosaic.

[c] NASHA = nucleic acid spot hybridization assay; + = positive reaction and − = negative reaction using a TMoV-specific probe in the NASHA.

infected Florida Lanai plants in a 48-hr acquisition access period. Whiteflies placed on test plants were given a 48-hr inoculation access period. At the end of the inoculation access period, all test plants were sprayed thoroughly with a 1% solution of insectidical soap (M-Pede, Mycogen Inc., San Diego, CA 92121), to kill the whiteflies. Test plants were then placed in screened cages. Control plants were caged separately from test plants. Symptoms were allowed to develop for 4 wk before leaf samples were collected and assayed by NASHA. Back inoculations were conducted the same as initial inoculations.

Nicotiana edwardsonii Christie & Hall and *N. benthamiana* Domin., which are difficult to inoculate with whiteflies, were inoculated using a mortar and pestle with a homogenate of young leaf tissue of TMoV-infected tomato plants and 0.01 M potassium phosphate buffer, pH 7.5, containing 1% beta mercaptoethanol.

Detection of TMoV in test plants. Young leaf tissue from control and test plants was collected and extracted immediately, or was kept frozen at −20 C until extraction for screening by NASHA to determine the host status of the plants. Tomato tissue and other succulent tissues were extracted using a leaf squeezer. A 10 μl sample was taken from the expressed sap and added to 80 μl of TE buffer (10 mM Tris, pH 8.0, and 1 mM EDTA, pH 8.0). Mucilaginous tissue, such as okra (*Abelmoschus esculentus* (L.) Moench) and *Sida acuta* J. Burm., were extracted by cutting three to four tissue disks out of leaves with a no. 4 cork borer (which was washed with 95% ethanol between samples). Disks were placed in 1.5-ml microcentrifuge tubes, each containing 70 μl of TE buffer, and were ground using a plastic micropestle. To both types of extracted and diluted sap, 26 μl of freshly made 1 M NaOH was added. The contents were mixed and incubated at room temperature for 10 min. Extracts were then treated with 26 μl of 3 M sodium acetate, pH 5.2. The contents were mixed and incubated at room temperature for 10 min. Extracts were centrifuged at maximum speed in a microcentrifuge for 5 min. Supernatants of the extracted samples were spotted in 15 μl aliquots onto TAE (40 mM Tris-acetate, 1 mM EDTA, pH 8.0) wetted Nytran membranes (Schleicher and Schuell, Inc., Keene, NH 03431) using a blotting manifold (Hybrid-Dot Manifold, Life Technologies, Inc., Gaithersburg, MD 20877). Membranes were air-dried and then baked at 80 C for 30 min.

TMoV DNA was detected using DNA of cloned full-length TMoV B component which was labeled with ^{32}P using an Amersham Megaprime labeling system (Amersham, Arlington Heights, IL 60005). This labeled probe was hybridized to the blots at 65 C overnight and

rinsed from the blots under high stringency conditions. Blots were exposed to X-ray film for 4–24 hr at −70 C.

RESULTS AND DISCUSSION

Seed transmission. No evidence of transmission either on the surface of the tomato seed or in the tomato seed was found. The sensitivity of the assay suggests that if seed transmission occurred, it would be at a rate less than 0.03%. These findings are similar to those of a study of chino del tomate virus in which no seed transmission was found in 300 tomato seeds (3).

Symptomatology and host range. A total of 41 species belonging to 24 genera in eight families were tested for their susceptibility to TMoV. TMoV was found to infect primarily plants belonging to the Solanaceae (Table 1). Fourteen species in this family were tested and nine became infected within 3 wk of inoculation. Of the seven solanaceous genera inoculated, only three, *Lycopersicon, Nicotiana,* and *Physalis,* could be infected. TMoV caused the following

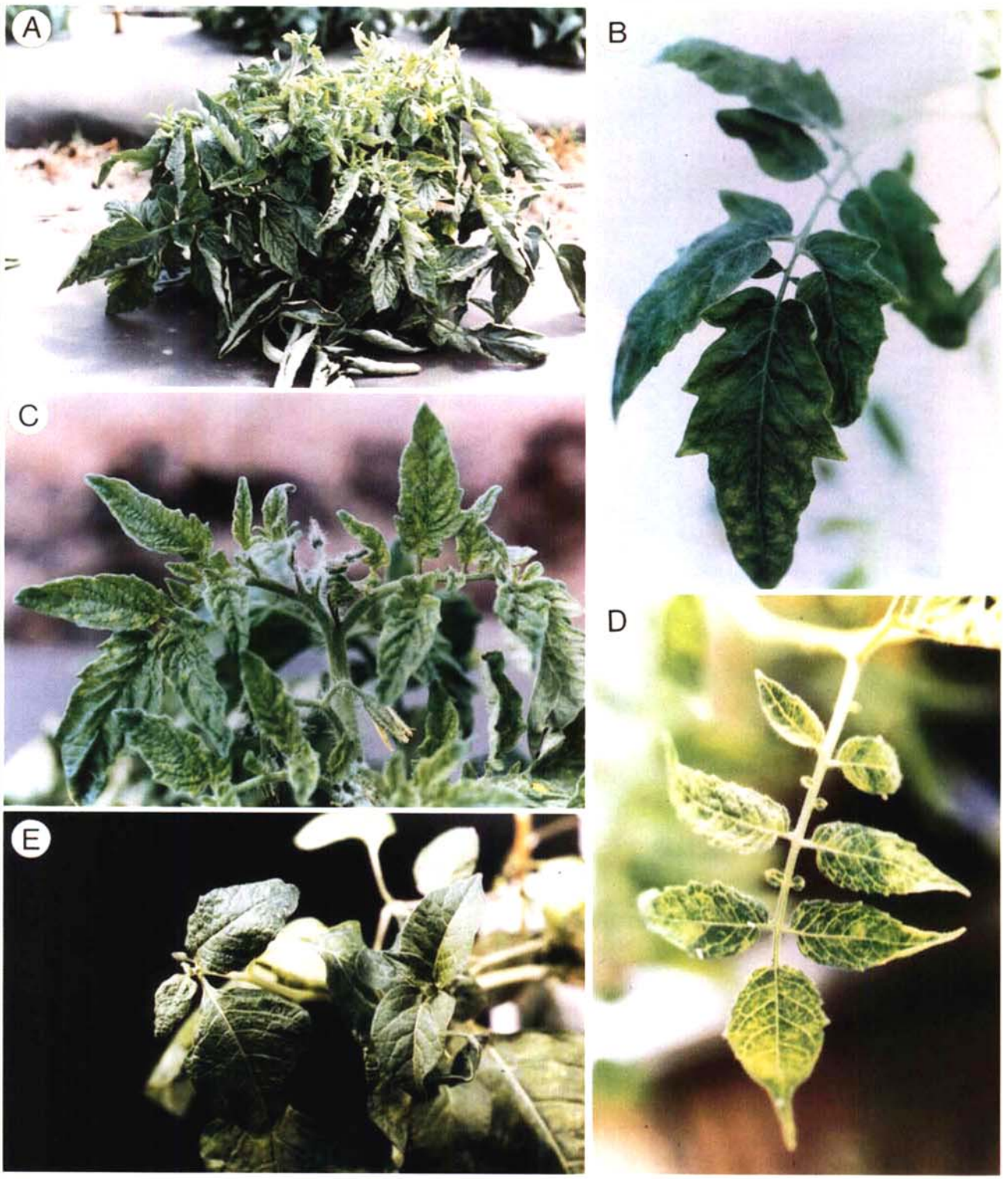

Fig. 1. Plants showing symptoms of tomato mottle virus (TMoV) infection. **(A)** 8-wk-old cv. Sunny tomato plant, naturally infected with TMoV, showing upward leaf curling and stunting. **(B)** Leaflet of a greenhouse grown Sunny tomato plant inoculated by whiteflies with TMoV showing mottling of leaflets. **(C)** Close-up of 8-wk-old Sunny tomato, naturally infected with TMoV, showing mottling of young leaflets. **(D)** Leaflet of greenhouse grown *Lycopersicon hirsutum* f. *glabratum* whitefly-inoculated with TMoV showing bright yellow mosaic. **(E)** TMoV inoculated *Physalis alkekengi* on the left, showing distortion and downward curling of leaves, and uninfected plant on the right.

symptoms in *L. esculentum*: chlorotic mottling in the upper leaves, upward curling of middle and lower leaves, and an overall reduction in plant height (Figs. 1A–C). Older leaves of infected tomato plants often had either a chlorotic mottle or mosaic, and upwardly rolled leaflets. Early infections resulted in more severe stunting. Infections of young tomato seedlings produced deformed leaves with a faint mottle. The symptoms described from greenhouse-inoculated tomato plants were similar to those seen in the field in naturally infected commercial tomato cultivars (e.g., Sunny, Agriset 761, and Solar Set). The symptoms in other *Lycopersicon* species were a bright yellow mosaic, often accompanied by a downward curling of the leaves (Fig. 1D).

All three species of *Physalis* which were inoculated became infected and displayed various symptoms. Infected plants of Chinese lantern (*P. alkekengi* L.) were stunted and had a pronounced downward leaf curling (Fig. 1E). Infected plants of tomatillo (*P. ixocarpa* Brot. ex DC.) were stunted and displayed a bright yellow mosaic. Infected plants of Wright groundcherry (*P. wrightii* A. Gray), a solanaceous weed in the southwestern United States, were stunted and had puckered, mildly distorted leaves with an interveinal chlorosis. The symptoms in *Nicotiana* species were a mosaic with a downward curling of leaves.

Common bean (*Phaseolus vulgaris* L. 'Topcrop'), a legume host of many whitefly-transmitted geminiviruses (2,4, 5,7,14), was the only legume species out of 10 tested that became infected with TMoV. No symptoms of virus infection were seen on Topcrop plants which were positive by NASHA, although whiteflies were able to acquire and subsequently transmit TMoV from these plants.

TMoV could be readily distinguished by host range from other whitefly-transmitted geminiviruses found in Florida. One other geminivirus, pseudo-curly top (PCTV), occurs naturally in tomato in Florida (12,18). However, PCTV is a treehopper-transmitted geminivirus and causes severe distortion, stunting, and leaf curling in tomato. Macroptilium golden mosaic virus-Florida (MGMV-FL) (originally named bean golden mosaic virus-Florida), Sida mosaic virus-Florida, bean golden mosaic virus-Florida (BGMV-FL), and a Brassica-infecting geminivirus are all transmitted by *B. tabaci* but do not infect tomato and do infect plants which are not hosts of TMoV (7; J. Strandberg, E. Hiebert, G. L. Leibee, and A. Abouzid, *unpublished*; J. E. Polston, *unpublished*). TMoV is unique among the other geminiviruses described from Florida based on this host range.

Partial and extensive host ranges for seven whitefly-transmitted geminiviruses which can infect tomato have been reported from the Western Hemisphere. TMoV could be distinguished from each of these viruses based on the host range, and in some cases on symptomatology alone. Serrano golden mosaic virus from Arizona and Mexico was able to infect pepper and jimson weed, *Datura stramonium* L., while TMoV could not (5). Tomato yellow mosaic virus from Venezuela (TYMV) infected *D. stramonium* and petunia (*Petunia* × *hybrida* Hort. Vilm.-Andr.), did not infect common bean (*P. vulgaris*), and elicited a bright golden mosaic in infected tomato (6). Tomato golden mosaic virus from Brazil (TGMV) elicited a bright golden mosaic in tomato and could infect *D. stramonium,* unlike TMoV (11). The Texas pepper geminivirus infected bell pepper (*Capsicum annuum* L.) and *D. stramonium* but did not infect common bean (19). Chino del tomate (CdTV) infected *D. stramonium,* mungbean (*Vigna radiata* (L.) R. Wilcz.), and bell pepper, while TMoV did not (4). Merremia mosaic (Puerto Rico) infected *D. stramonium,* scarlet runner bean (*Phaseolus coccineus* L.), tepary bean (*P. acutifolius* A. Gray), and lima bean (*P. limensis* Macfady) (2), which TMoV did not. TMoV could be distinguished from potato yellow mosaic virus (PYMV) because the latter infected potato (*Solanum tuberosum* L.) and petunia, while TMoV did not (15). The host range and nucleic acid sequence of TMoV (1) suggest a distinct virus, although it is unclear at this time how host range differences should be used to classify geminiviruses as different viruses or different strains of the same virus. Minor sequence differences between two strains of squash leaf curl virus were reflected in host range differences at the host genus level (10,13). However, a comparison of the genome sequences among TGMV, PYMV, and TMoV strongly suggested that TMoV is significantly different from these tomato-infecting geminiviruses, as well as from four other whitefly-transmitted geminiviruses from the Western Hemisphere, Abutilon mosaic, BGMV-FL, BGMV-PR, and squash leaf curl virus (E strain), which do not infect tomato (1,2,10,14; J. E. Polston, *unpublished*).

This host range has significant implications for the management of TMoV in Florida. Two other solanaceous crops, bell pepper and potato, are grown in large acreages in the same areas and in overlapping production cycles with tomato. The inability of TMoV to infect these crop plants eliminates them as potential sources of TMoV for the epidemics in tomato. Other potential hosts, of lesser concern because of their reduced frequency in tomato production areas, were *A. esculentus* (okra), *Solanum melongena* L. (eggplant), *E. heterophylla, M. lathyroides,* and *D. stramonium.* Studies are in progress to identify wild plant hosts which may be contributing to TMoV epidemics in tomato.

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